A harm-reduction approach to the isolation of harmine and its hydrogenated derivatives from Peganum Harmala seed in low-tech settings

Frederick A.P. Van Der Sypt, M.D.

Abstract

Background. Peganum Harmala (Syrian rue, Nitrariaceae) has a long and widespread history of ethnopharmacological use. Due to its rich and diverse alkaloid content, the traditional application of whole plant material or full alkaloid extract is inherently unspecific and therefore susceptible to side effects. Simultaneously, incorrect phytochemical procedures for its use in ayahuasca-analogues cause intoxications in misinformed users.
Aim of the study. Providing harm-reduction by developing easily applicable, safe and effective isolation protocols for harmine, harmaline (dihydroharmine) and tetrahydroharmine (leptaflorine) from Peganum Harmala seed.
Materials and methods. Only commonly available equipment and reagents were used for isolating the alkaloids. Following extraction, harmine and harmaline were separated using 2 low-tech methods (selective precipitation using sodium bicarbonate or pH-metry). Then, zinc-acetic acid reduction of both harmine and harmaline was attempted. The identity and purity of the obtained alkaloids were confirmed using microscopy and melting point determination.
Results. Using pH-metry for the separation of harmine and harmaline proved rapid, effective and recovered 91% of alkaloids. Selective precipitation of the alkaloids with sodium bicarbonate had a yield of 76%. Hydrogenation of harmaline to tetrahydroharmine using zinc-acetic acid had a yield of 83%. Harmine however could not be hydrogenated by zinc-acetic acid. The melting ranges of the obtained alkaloids were narrow and consistent with literature. Based on these results, isolation protocols were developed.
Conclusions. Harmine, harmaline and tetrahydroharmine can be isolated with good yields from Peganum Harmala seed following protocols that are applicable in traditional and other low-tech settings. This study showed zinc-acetic acid to be effective in the reductive synthesis of tetrahydroharmine and also proposes a rapid, precise and high yielding method for the bulk separation of harmine and harmaline using basic pH-metry.

1.Introduction

Harmine (banisterine, telepathine, yageine), dihydroharmine (DHH, harmaline, harmidine) and tetrahydroharmine (THH, leptaflorine) are β-carboline alkaloids that have mutiple organic sources, geographical regions of medicinal use and etnopharmacological applications. An important biological reservoir of these alkaloids is Peganum Harmala (Syrian rue, Nitrariaceae). Since ancient times, it has been applied by many cultures in India, Pakistan, the Middle East and North Africa as an abortifacient, cicatrizans, aphrodisiac, stimulant, sedative, antispasmodic, narcotic, analgesic, antibiotic, anthelmintic, antipyretic as well as in the treatment of rheumatism, diarrhea, inflammation, hypertension, depression, diabetes, cancer and astma [Bensalem et al., 2014; Moloudizargari et al., 2013; Asgarpanah & Ramezanloo, 2012; Patel et al., 2012].

Harmine, DHH and to a lesser extent THH are found to account for most of the plant's psychotropic effects [Moloudizargari et al., 2013; Khan et al., 2013; Patel et al., 2012; Herraiz et al., 2010]. However, many other alkaloids are present and exert wholly different effects. E.g. the abortifacient properties of the plant have been attributed to vasicine and vasicinone (quinazoline alkaloids)[Moloudizargari et al., 2013]. Because traditional medicine mainly employs full alkaloid extracts or whole plant parts, the pharmacological effect is inherently unspecific and the potential for side-effects is ever-present. In spite of their long traditional use, surprisingly few data is available describing the isolation of the individual alkaloids. This present study aims to provide information on the isolation of the aforementioned alkaloids following protocols that are applicable in traditional, low-tech settings. Hereby, it hopes to provide some reciprocity in practical phytochemical knowledge and to make the traditional use of the plant more specific and safe.

At the same time, this study has a more contemporary objective as well. In recent years, a global 'psychedelic reanaissance' has emerged, where 5-HT2A receptor agonists with psychotropic action are being explored for multiple somatic [Herrendorff et al., 2016; Spindler A, Stefan K, Wiese M, 2016; Sun K, Tang XH, Xie YK, 2015] and psychic conditions such as depression and

addiction [MAPS, 2016; Nunes et al., 2016; Frecska et al., 2016; Dos Santos et al., 2016a; Dos Santos et al., 2016b]. One such psychedelic under study is the entheogenic brew ayahuasca. In the wake of ayahuasca's growing popularity, the use of the aforementioned β-carboline alkaloids as MAO-inhibitors for the activation of oral tryptamines in ayahuasca-analogues is growing as well [Herraiz et al., 2010]. Particularly the use of Peganum Harmala seed is discussed on various websites because of its rising (online) availability and low cost [Dmt-nexus 2016a; Erowid 2016a; Mycotopia 2016; Shroomery 2016]. To bypass dosing difficulties, nausea and more serious side-effects, users try to isolate harmine and DHH from the seeds. Also, some users have tried hydrogenating DHH to THH using vinegar and zinc-powder [Dmt-nexus 2016b]. Upon reading the techniques for alkaloid extraction, purification and conversion on these popular sites, many are observed to be chemically incorrect. This is a real cause of intoxications for misinformed users [Frison et al., 2008; Brush DE, Bird SB, Boyer EW, 2004; Boyer EW, Shannon M, Hibberd PL, 2001]. Most of the time, the availability of technical equipment for these ayahuasca-analogue users is comparable to traditional settings. Therefore, the development of safe and effective low-tech protocols can simultaneously provide harm-reduction for these risk-groups as well. This present study will try to answer this need by placing the emphasis not only on the availability of reagents, but also on their inherent safety.

Modern extraction and purification techniques of Peganum Harmala plant material mainly use hazardous solvents and sophisticated equipment [Pulpati H, Biradar YS; Rajani M, 2008; Monsef-Esfahani et al., 2008; Kartal M, Altun ML, Kurucu S, 2003]. These protocols aim for complete extraction of the plant material for identification and quantification purposes. However, if the goal is only to extract and isolate the aforementioned β-carboline alkaloids, some of their specific characteristics make it possible to apply a low-tech approach. Pioneering work in this field has already been done and described at length by Hasenfratz [Hasenfratz, 1927]. This current study will build upon his research and successively investigate the pH-specific separation of alkaloids, the use of sodium bicarbonate as an aid in separation, the conversion of DHH to THH through reduction with zinc-acetic acid and the possibility of reducing harmine to THH using zinc-acetic acid. Each time, the identity and purity of the end-products will be confirmed by accessible techniques.

2. Methods and materials

2.1 Crude alkaloid extraction from seeds

A batch of Iranian Peganum Harmala seeds with number ET-1545 was obtained from an internet supplier, Gaiana ethnobotanicals. To 251,9g of whole seeds, 500ml of water and 500ml of vinegar 7% were added. The seeds were left to sit in the solution for 12 hours. Not much swelling was noted.

Then the solution was boiled for 1 hour under regular stirring. A lot of liquid was absorbed by the seeds. The mass was filtered through cheesecloth, and 165ml of vinegar 7% and 335ml of water were added to the seeds. The mixture was again boiled for 1 hour and strained through cheesecloth. A third extraction cycle was executed in the same manner.

The fourth time, after adding the acid and water, the solution was put in a blender, effectively grinding the seeds. These ground seeds were boiled for 1 hour and filtered. The resulting seed mash then was discarded.

A batch of sodium carbonate was synthesized by heating sodium bicarbonate at 200°C for 2 hours. To the first extract, sodium carbonate was added under stirring. An excess of base was added until the pH stabilized at 9,6. The solution was left to stand for 12 hours, filtered first through cotton swabs and then through regular filter paper using a vacuum-filtration setup.

The filtrates of the second, third and fourth extraction were treated in the same way with an excess Na_2CO_3. The precipitated alkaloids were filtered, dried and weighed.

2.2 Separation of harmine and DHH from the other alkaloids

The total mass of alkaloids was dissolved in 125ml of hot vinegar 7% and this solution was left to stand for 6 hours to let the fine plant material settle. Then the supernatant was filtered through fine filter paper and reheated. In another recipient, 88g of NaCl was dissolved in 250ml of boiling water and this solution was added to the hot extract. The mixture was then allowed to cool slowly for 12 hours.

In the course of cooling, the hydrochlorides of harmine and DHH selectively recrystallized as long golden needles. These were filtered, redissolved in 200ml of demineralized water and heated to near boiling. In another recipient, 70g of NaCl were dissolved in 200ml of boiling water. This solution was added to the hot alkaloid solution and again, the solution was left to cool slowly for 12 hours. In the meantime, an excess of Na_2CO_3 (30g) was dissolved in the filtrate to precipitate the other alkaloids. These were left to settle in a tall recipient for inspection and comparison with following precipitates.

Again, the alkaloids were filtered, redissolved and treated with NaCl-solution in the same manner as above and left to recrystallize. The filtrate was basified and the amount of precipitate was inspected after sedimentation. This same protocol was followed a fourth time.

The crystal mass that was obtained from the fourth recrystallization was dissolved in 200ml of hot water and left to stand for 12 hours before filtering the solution through fine filter paper (8-12µ). A final time, hot saturated sodium chloride solution was added as above and after 12h, the mixed harmala-hydrochlorides were filtered. The filtrate was basified and left to stand for inspection of the precipitate.

Next, the hydrochlorides were converted to their freebases: the dried and NaCl-contaminated mass of crystals from the last recrystallization step was dissolved in 200ml of hot water and an excess of Na_2CO_3 was added until the pH stabilized at 9,75. The precipitated freebases were filtered and washed by stirring in 100ml ammonia solution 3% for three times. The filtrates of these washings were left to evaporate for inspection to evaluate the losses associated with the ammonia-washings.

2.3 pH specific precipitation and separation of harmine and DHH freebase

6004mg of mixed harmala-freebase as obtained from experiment 2.2 were dissolved in 250ml of demineralized water to which 35ml of acetic acid 7% were added. Under continuous monitoring of the pH with a Voltcraft PH-100 ATC, an ammonia 1% solution was added in small increments. When precipitation was observed, the solution was filtered and the residue was left to dry. Because crystals attached themselves to the sides of the reaction vessel, it was rinsed with

vinegar after filtration and the freebase was precipitated from the washing solution with an excess ammonia and the dried residue added to the main filtered fraction. Then, the addition of ammonia to the filtrate was continued. Following each filtration, a tiny amount of freebase was observed under a basic microscope (Bresser Bio Discover 20x-1280x) and again after dissolving it on the microscopic slide in a drop of acetic acid and reprecipitating it *in situ* with a drop of ammonia 12%.

A total of 19 precipitated fractions were isolated and examined microscopically. In the course of the experiment, the pH and the cumulative amount of precipitate were plotted against the amount of base added.

Next, fractions 1-10 were homogenously mixed, washed with ammonia 3% solution, filtered and left to dry, giving fraction RA1. This fraction was redissolved in 4ml of acetic acid 7% and 6ml of water, precipitated with 10ml of saturated NaCl-solution, filtered, redissolved in water, precipitated with ammonia 12% and washed 3 times with ammonia 3%. This residue was then dried to give RA2. A small sample of this fraction was consecutively sublimated at 1mbar and 150°C: RA7.

Then, fractions 13-19 were mixed as well, washed with ammonia 3%, filtered and left to dry: fraction RA3. This fraction was dissolved in acetic acid and reprecipitated with ammonia 12% giving fraction RA4. Lastly, fraction RA4 was sublimated at 1mbar and 150°: RA5.

After filtering fraction 19, the crystals that were attached to the recipient were redissolved, precipitated with ammonia 12%, washed and dried to give residue RA6. After examination, this residue was added to RA3.

In line with a low-tech approach, the melting ranges of all labeled residues were determined using a Thiele tube. The melting ranges of three consecutive samples of each residue were measured. These results were averaged and rounded to the nearest half degree.

2.4 Using sodium bicarbonate as a base for precipitation

1940mg of mixed harmala-alkaloids were dissolved in 38ml of water to which 12ml acetic acid 7% were added. Under stirring, there was added a solution of 8g $NaHCO_3$ in 100ml of water. When 30ml were added, precipitation started. The remaining 70ml of solution were then added as well. The suspension was allowed some time to crystallize before it was filtered to give residue RA1. This was washed in the filter with 3x30ml of Na_2CO_3 0,5%, dried and weighed. The pH-curve of this first precipitation with $NaHCO_3$ was drawn.

Then, under stirring, 4g of Na2CO3 were added to the filtrate. Precipitation ensued. After filtering, the residue RA2 was washed with 3x15 ml of Na_2CO_3 0,5%, dried and weighed. This completed the first precipitation cycle.

Now residue RA1 was dissolved in 10ml acetic acid 7% + 40ml of water. Again, 100ml of $NaHCO_3$ 8g/100ml was added under stirring. Precipitate RA3 was filtered, washed with 3x30ml of Na_2CO_3 0,5%, dried and weighed.

Then 4g of Na2CO3 were dissolved into the mother liquor. Precipitation ensued. After filtering, the residue RA4 was washed with 3x15 ml of Na_2CO_3 0,5%, dried and weighed. This completed the second precipitation cycle.

Three more cycles were done following the above steps, thereby generating RA5-RA10. All residues were weighed and for every cycle, the $NaHCO_3$ and Na_2CO_3 fractions were visualized in a graph.

The melting ranges of residues RA9 (harmine fraction), RA2 and RA8 (first and last DHH-fraction) were determined using a Thiele tube. The melting ranges of three consecutive samples of each residue were measured. These results were averaged and rounded to the nearest half degree.

2.5 Conversion of DHH freebase to THH

2002mg of DHH was dissolved in 100ml of acetic acid 7%. To this solution, 3008mg zinc powder was added. This mixture was left to stand at room temperature for several hours under regular stirring. It was observed under UV-light that the solution's fluorescent color changed from yellow-green to deep blue in the course of 3 hours. The mixture was left to react for an additional 6 hours. Then it was filtered giving residue RA1 and the recipient was washed with water that was poured over the unreacted zinc in the filter and was thereby added to the filtrate. This brought the total volume of filtrate to 200ml.

Under continuous pH-measurement, a solution of ammonia 12% was added dropwise. The starting pH was 3,88. When the pH reached 5,42 after adding 16ml of base, a very fine mist appeared in the solution that did not augment during the further addition of base. At pH 7,25 (21,5ml of ammonia added in total), the solution was filtered again to give residue RA2.

More ammonia was added and after the total addition of 33ml of ammonia 12% (pH 8,77), clouding once again appeared in the solution and the pH declined steadily to 8,62. At this point the solution was filtered again and precipitate RA3 was obtained.

The addition of ammonia was continued, but no more clouding nor pH-depression was observed up to a pH of 9,64. At that moment, 4ml of ammonia 12% were added that gave rise to clouding at a pH of 9,76. The solution was filtered again to give residue RA4.

A small amount of RA4 was then sublimated at 1mbar and 130°C to give RA5. Another amount of RA4 was recrystallized twice from ethanol 96% giving RA6.

Then an excess (20ml) of ammonia 12% solution was added that brought the pH to 10,02. No precipitate formed.

The melting ranges of three consecutive samples of each residue were measured using a Thiele tube. These results were averaged and rounded to the nearest half degree.

2.6 Hydrogenation of harmine by zinc-acetic acid

2 experiments were conducted simultaneously:

Experiment 2.6.1

1000mg of harmine from residue RA2 obtained in experiment 2.3 was dissolved in 50ml of acetic acid 7% and left to sit at room temperature for 24h. Then, ammonia 12% was gradually added. When 9ml of base were added, the solution turned cloudy. After adding 1ml more, a lot of precipitation ensued. The addition of another 3ml of ammonia did not provoke any further precipitation. The solution was filtered, washed with 3x20ml of ammonia 3% and left to dry (RA1).

Experiment 2.6.2

1002mg of harmine from the same batch as experiment 2.6.1 was dissolved in 50ml of acetic acid 7%. Then 1,5g of zinc powder was added and the solution was left to react at room temperature for 24h. The unreacted zinc was filtered off (RB1) and ammonia 12% solution was slowly added to the filtrate. After having added 7,5ml of ammonia, some flocculent precipitate was observed in the solution and adding 0,5ml more did not increase this amount. The solution was filtered, the residue washed with 3x20ml of ammonia 3% and dried (RB2). More ammonia was added to the filtrate. At 9ml, obvious precipitation started and the solution was filtered, the residue washed with 3x20ml of ammonia 3% and dried (RB3). Further dropwise addition caused immediate precipitation and after adding 0,7ml more, no further precipitation was noted. Again the solution was filtered, the residue washed with 3x20ml of ammonia 3% and dried (RB4). Then an excess of ammonia 12% was added to the filtrate that caused no more precipitation.

Precipitates RA1 and RB2-RB4 were examined microscopically.

The melting ranges of three consecutive samples of each residue were measured using a Thiele tube. These results were averaged and rounded to the nearest half degree.

2.7 Zinc-acetic acid reduction of mixed harmine-DHH freebases and pH-specific separation with ammonia, $NaHCO_3$ and Na_2CO_3

3,002g of mixed harmala-freebases from experiment 2.2 were dissolved in 100ml of acetic acid 7%. To this solution, 3g of zinc powder were added and the reaction vessel was left to stand at room temperature for 17 hours. Then, the solution was filtered and the unreacted zinc was discarded. After washing, the total volume of filtrate was 159ml (A).

To 53ml of this solution (A1), ammonia 12% solution was progressively added until the pH read 7,75 (7ml was needed). At that moment, pH-depression and clouding of the solution was observed. The suspension was filtered to give residue R1A1. To the filtrate more ammonia was added until clouding and pH-depression was again observed at pH 9,45 (6ml was needed). The suspension was once again filtered to give residue R2A1. Now, 15ml of ammonia 12% were added to the filtrate that brought the pH to 10,15. No clouding or pH-depression was observed. Residue R1A1 was further purified first by dissolving it in acetic acid and adding an equal volume of saturated NaCl-solution. The reactants were filtered, the crystals redissolved in water and precipitated with ammonia 12%. The precipitate was filtered off and dried to give R3A1.

To the remaining 106ml of filtrate A (A2), 4g of $NaHCO_3$ were added. When adding the fifth gram of $NaHCO_3$, clouding was observed. 75ml of water and an excess (12g) of $NaHCO_3$ was added.That stabilised the pH at 7,25. The suspension was filtered to give residue R1A2 and filtrate B (290ml).

To half of B (145ml), B1, 3g of Na_2CO_3 was added which provoked clouding and precipitation and brought the pH to 9,45. 150ml of water and an excess (10g) of sodium carbonate were added and stabilized the pH at 9,89. The suspension was filtered to give R1B1.

To the other half of B (B2), 50ml of water was added. Then, ammonia 12% was added in small increments. At 10ml, pH 9,52 was reached and pH-depression was noted to 9,40. Then, an additional 20ml of base was added to stabilise the pH at 10,03. After filtration, residue R1B2 was obtained and dried. 100mg of R1B2 were converted to the hydrochloride salt with hydrochloric acid and the melting range of the resulting yellow crystals R2B2 was determined.

Residue R1A2 was purified in 2 different ways.

1) 990mg of the total 1974mg were dissolved in 23ml water + 17ml vinegar 7%. To these 40ml of solution, 40ml of saturated NaCl-solution were added. The precipitate was filtered, dried and redissolved in 30ml of water. The, 20ml of ammonia 12% were added. The suspension that formed was filtered, the residue washed 3 times with 5ml of ammonia 3% and dried. Thus was obtained residue R2A2.

2) The remaining 984mg were dissolved in 20ml of vinegar 7% + 100ml of water. Then, 30ml of ammonia 12% were added. The precipitate was filtered and dried to yield residue R3A2.

The melting ranges of three consecutive samples of each residue were measured using a Thiele tube. These results were averaged and rounded to the nearest half degree.

2.8 Disposal of reactants

When all experiments had been conducted, the reactants were destroyed by pyrolysis.

3. Results

3.1 Crude alkaloid extraction from seeds

Working in the outlined manner, the following amounts of dry precipitate were obtained: extract 1: 5673mg; extract 2: 7038mg; extract 3: 3445mg; extract 4: 2802g. The absorption of extraction fluid by the seeds during the first extraction accounts for the paradoxical rise in yield during the second cycle.

This brought the total amount of crude extracted freebase alkaloids after 4 extraction cycles to 18,953g. Clearly, there were still alkaloids present in the seeds after the last extraction cycle.

3.2 Separation of harmine and DHH from the other alkaloids

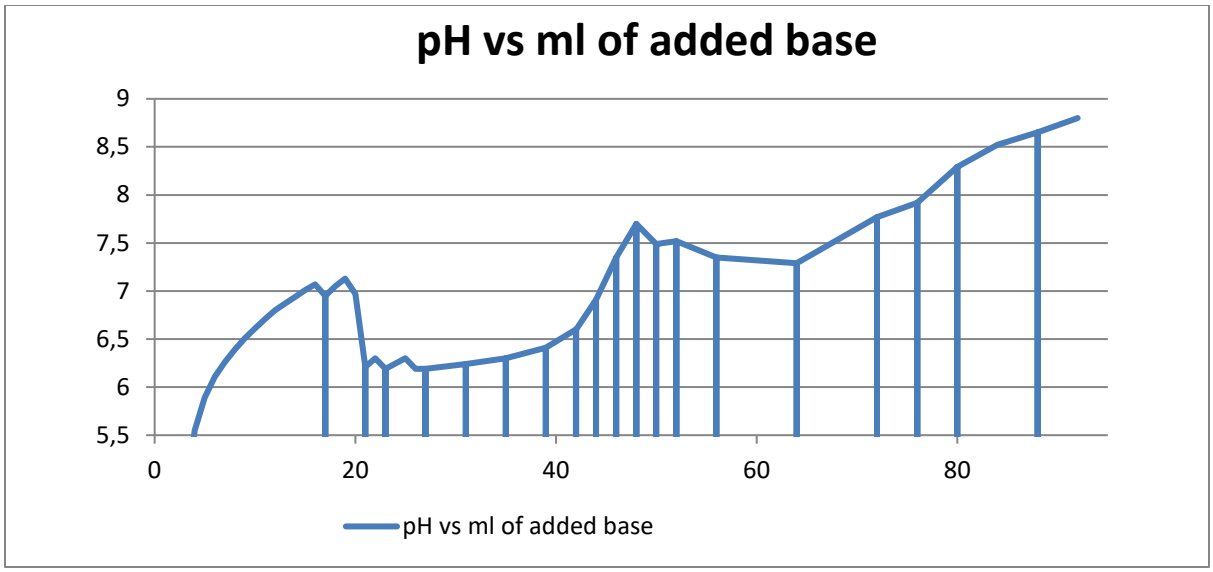
Throughout the sequential recrystallisation steps, it was observed that the amount of precipitated non-harmine/non-DHH freebase in the filtrates steadily declined over the course of the recrystallisation steps, getting very small and stable after the fifth.

The final harmine- and DHH-freebases were left to dry and weighed 36,4g or 48% of the total extracted alkaloid mass.

The filtrate of the third ammonia-washing evaporated without leaving any residue.

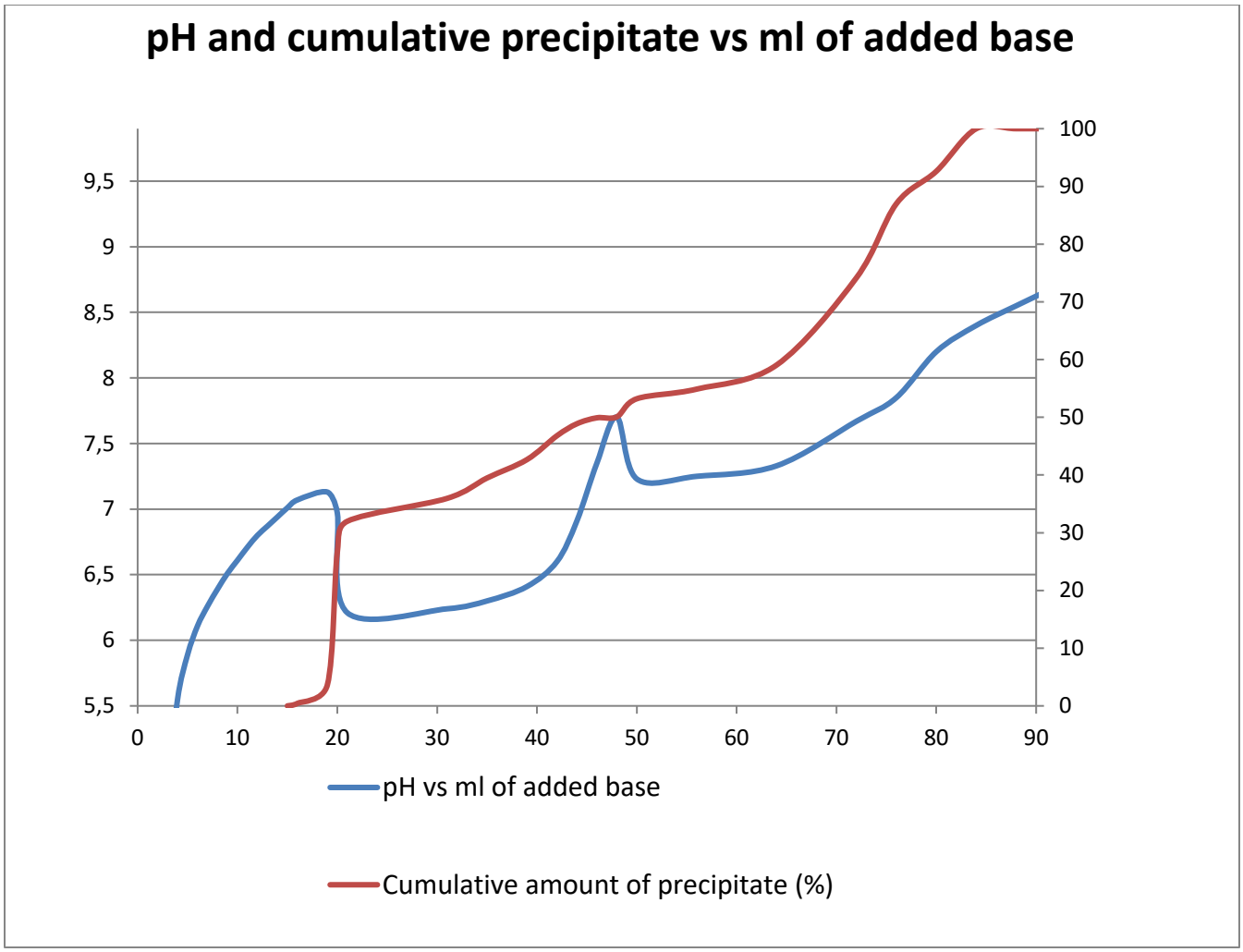
3.3 pH specific precipitation and separation of harmine and DHH freebase

Graph 3.3.1 shows the variation of pH during the addition of ammonia. The vertical lines represent the times when the solution was filtered to provide fractions 1-19. The spikes that appear in the curve are artefacts caused by removal of the precipitated alkloids. After filtration, the pH had to overcome an 'activation'-level before precipitation resumed.



Graph 3.3.1 The evolution of the mother liquor's pH during the selective precipitation of harmine and DHH. The vertical lines indicate the moments at which precipitate was filtered off.

Graph 3.3.2 shows the same curve but corrected for the small pH-variations. Additionally, the cumulative amount of precipitate is plotted over this curve, visualizing the concurrent pH depression and precipitation of alkaloids. It can be seen that when the pH was brought to 7,15, a quick and substantial pH-depression to about pH 6,20 was measured. Meanwhile, precipitate formed. Each time ammonia was added, an initial rise in pH was observed, quickly followed by a pH-depression down to a to a stable point. The curve connecting these lower points rises slowly. Nearing pH 7, this curve gets very steep. In other words, additions of small increments of ammonia give rise to a relatively large and stable pH-rise. Indeed, the amount of pH-depression gets minimal, vanishing at about pH 7,50. Microscopically, only needle-shaped harmine crystals could be observed in all of the preceding fractions. As can be observed from **Image 1 and 2**, crystal form can vary substantially depending on the specific conditions during formation. **Image 2** shows the same harmine fraction as in image 1, but now reprecipitated *in situ* on the microscopic slide using acetic acid and ammonia. This method reveals the characteristic needle-shape of the harmine crystals. **Image 3** shows the microscopic appearance of fraction 11 where only harmine crystals could be observed. This indicates the specificity of separation of both alkaloids, even very close to the point where DHH will start precipitating.



Graph 3.3.2 shows the pH curve and the cumulative amount of precipitate during the progressive freebasing of a harmine-DHH mixture. At 20ml and 50ml of added base, one observes the concurrent precipitation of alkaloids and depression of the solution's pH. The pH-peak right before 50ml of added base intersects the precipitation curve at about 50%, indicating the relative composition of the mixture.

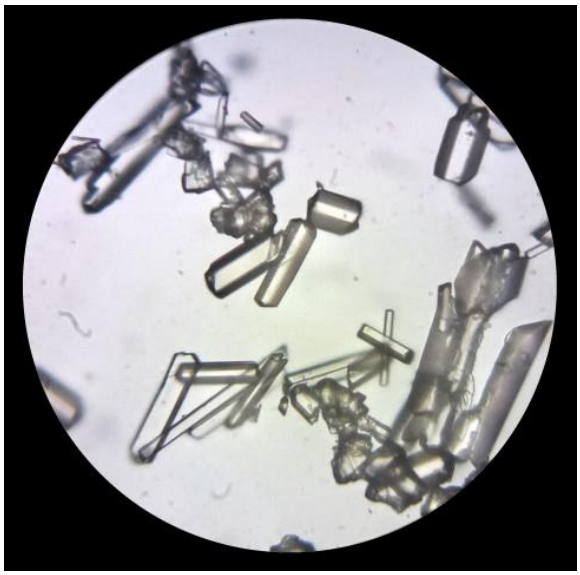


Image 1: Fraction 3: irregularly shaped harmine crystals are formed during gradual basification

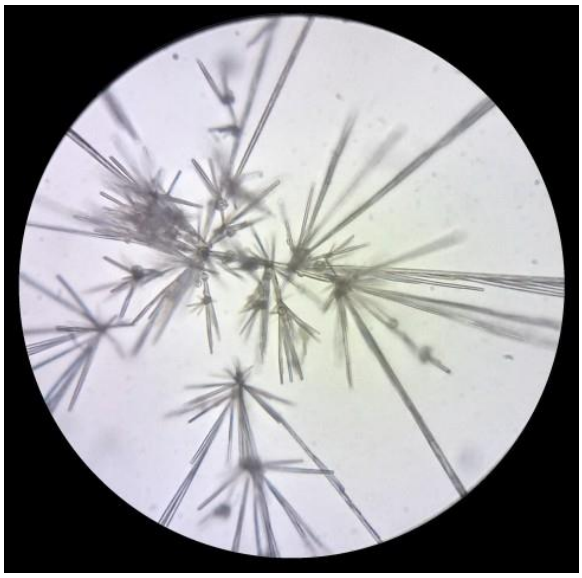


Image 2: Fraction 3, reprecipitated *in situ*. Now the characteristic needle-shaped harmine crystals can clearly be observed.

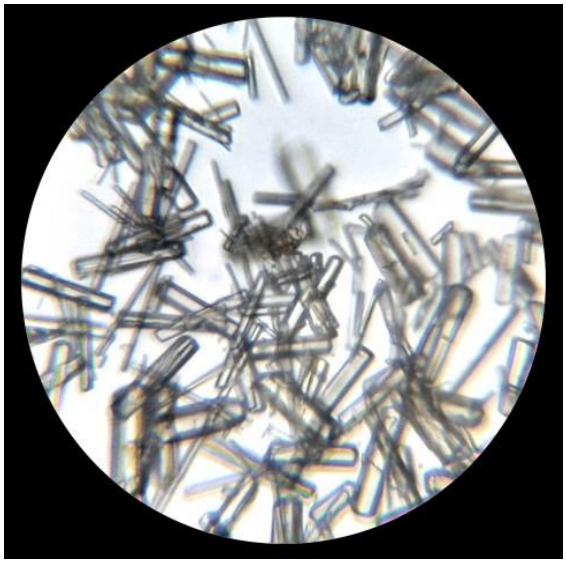


Image 3: fraction 11, showing only the presence of harmine crystals.

After rising to a peak at about pH 7,70, once again strong pH-depression was noted, together with clouding and precipitation. This fraction, fraction 12, when examined microscopically, contained largely DHH plates, but harmine crystals could be found too. This was observed microscopically in the originally precipitated sample ('sticks' and 'hexagonal plates', **Image 4**) as well as in the *in situ* reprecipitated sample ('needles' and 'agglomerated plates', **Image 5**).

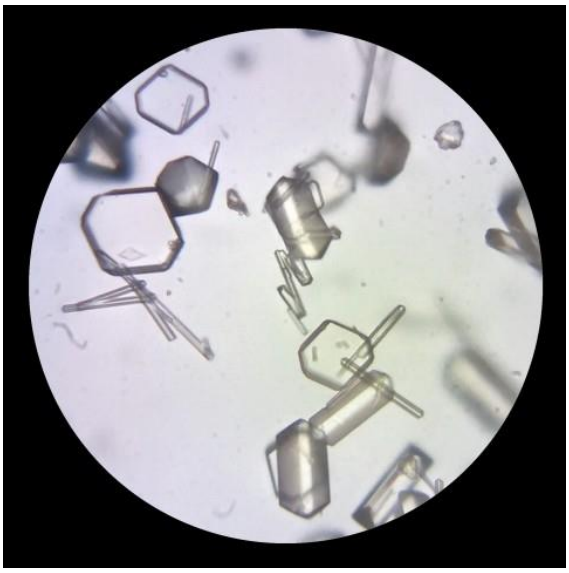


Image 4: the 12th or 'mixed-fraction' where both harmine 'sticks' and DHH 'plates' can be observed.

Image 5: the 12th or 'mixed-fraction', reprecipitated *in situ* on the microscopic slide.

When most of the harmine was filtered off, the precipitation of DHH could also be observed macroscopically by the appearance of flickering crystals (plates).

As can be observed in Image 5, impurities were attached to the crystals, possibly serving as nucleation sites.

A comparable pH-depression curve to the one of harmine, was now observed for DHH, with its lowest point at about pH 7,30. This curve rises as well and at pH 8,5 all of the DHH had precipitated. Addition of a large excess ammonia 12% solution did not lead to the formation of more precipitate. In fractions 13-19, no more harmine crystals were observed. **Image 6** shows fraction 13 as it was precipitated from the mother liquor. **Images 7** shows the same residue recrystallized *in situ* on the microscopic slide. Leaf-like crystals appear. **Image 8** shows another form of recrystallized DHH-precipitations that can be observed (fraction 16). Finally, **Image 9** shows the microscopic appearance of the last fraction that was precipitated. Here as well, only DHH was present and THH crystals could not be observed.

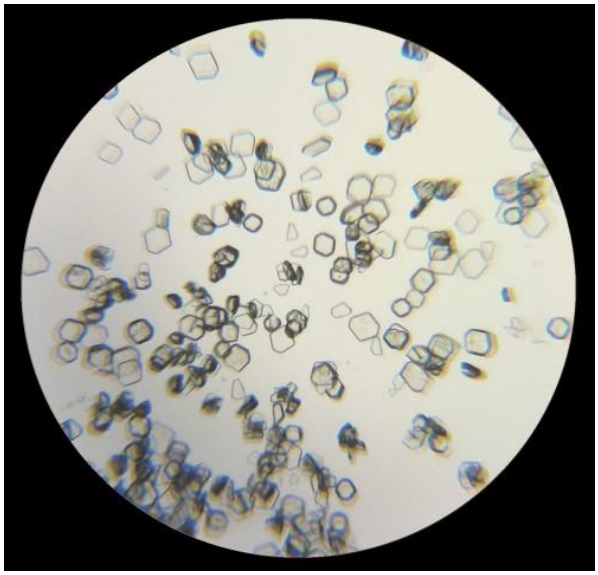


Image 6: Right after the mixed fraction, no more harmine can be detected in the precipitates. Here, in fraction 13, only harmaline plates are observed.

Image 7: fraction 13, recrystallized in situ on the microscopic slide

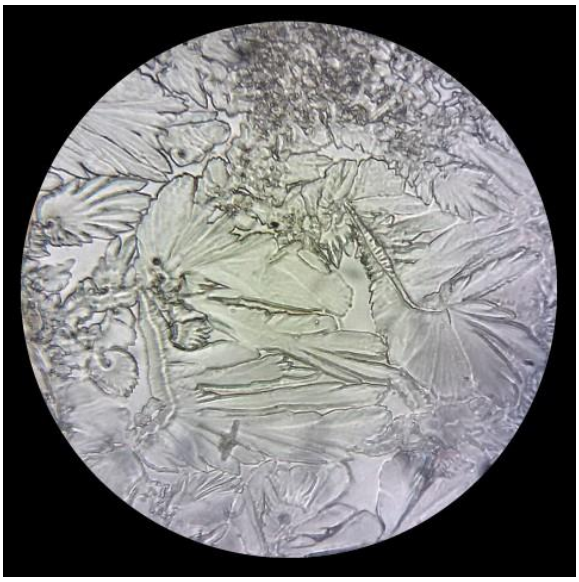
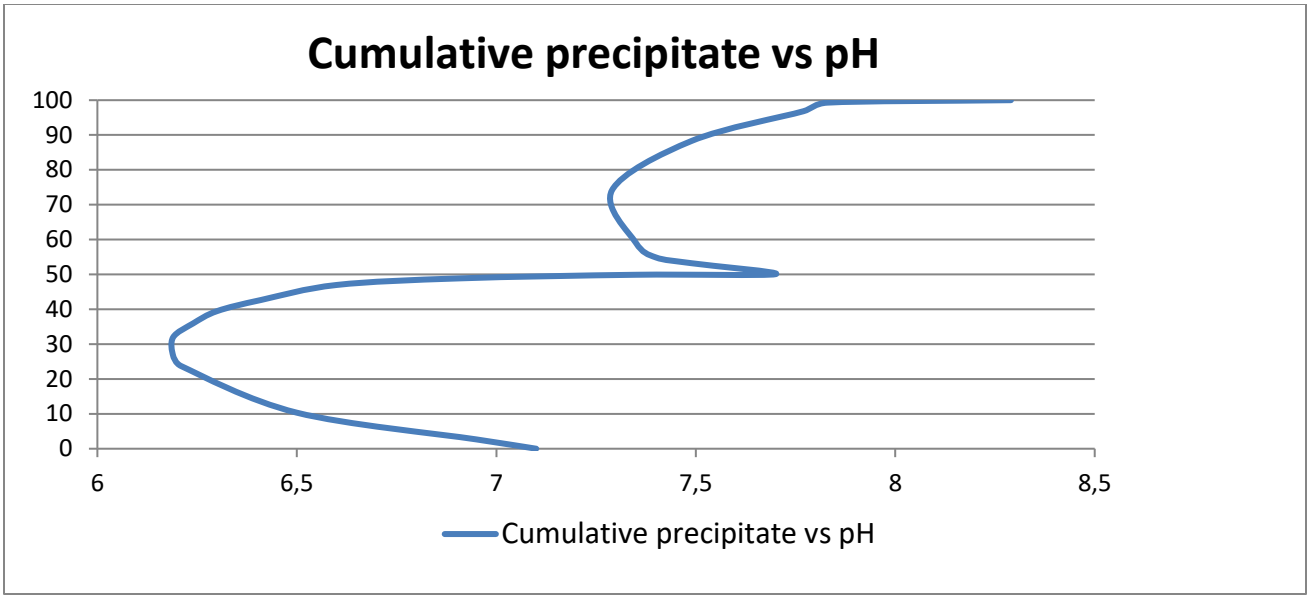


Image 8: Fraction 16: Next to the leaf-like crystals of image 7, other 'floral patterns' can be observed in recrystallized DHH-precipitates.

Image 9: Fraction 19: In the last fraction that was precipitated, THH crystals could not be observed next to the remarkable DHH-crystals

The graphic relationship between the cumulative amount of precipitate vs pH of the mother liquor can be observed in **graph 3.3.3**. It clearly shows the broad range the pH goes through when the solution nears depletion of harmine. When the addition of base is stopped at pH 7,2-7,5 the harmine fraction can be selectively removed with minimal loss. The subsequent mixed fraction can be kept to a minimum if it is filtered off right after the pH has peaked at about 7,7.



Graph 3.3.3 shows the amount of (cumulative) precipitate that was formed at a certain pH during the progressive freebasing of a harmine-DHH mixture.

In total, 5588mg (=93%) of alkaloids were recovered. Fractions 11&12 (mixed fraction) weighed 120mg (=2%). RA2 (harmine-only fraction) weighed 2678mg (48%), RA3 (DHH-only fraction) weighed 2790mg (50%). Fraction 19 weighed 32mg en was the final fraction to precipitate.

Sample	RA1	RA2	RA3	RA4	RA5	RA6	RA7
Melting range °C (decomp.)	253-255	256-257	227-229	237,5-238,5	239-240	235-237	258-259

Image 10 illustrates the one-step purification of the harmine fraction (RA2) by sublimation.

Image 11 shows sublimated DHH from fraction RA4.

Image 10: A fluffy and snow-white cluster of pure harmine-needles RA7 (left) sublimated from fraction RA2 (right).

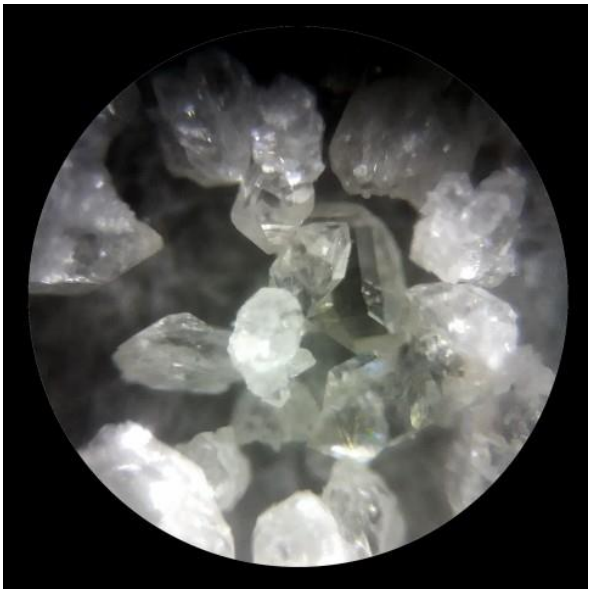
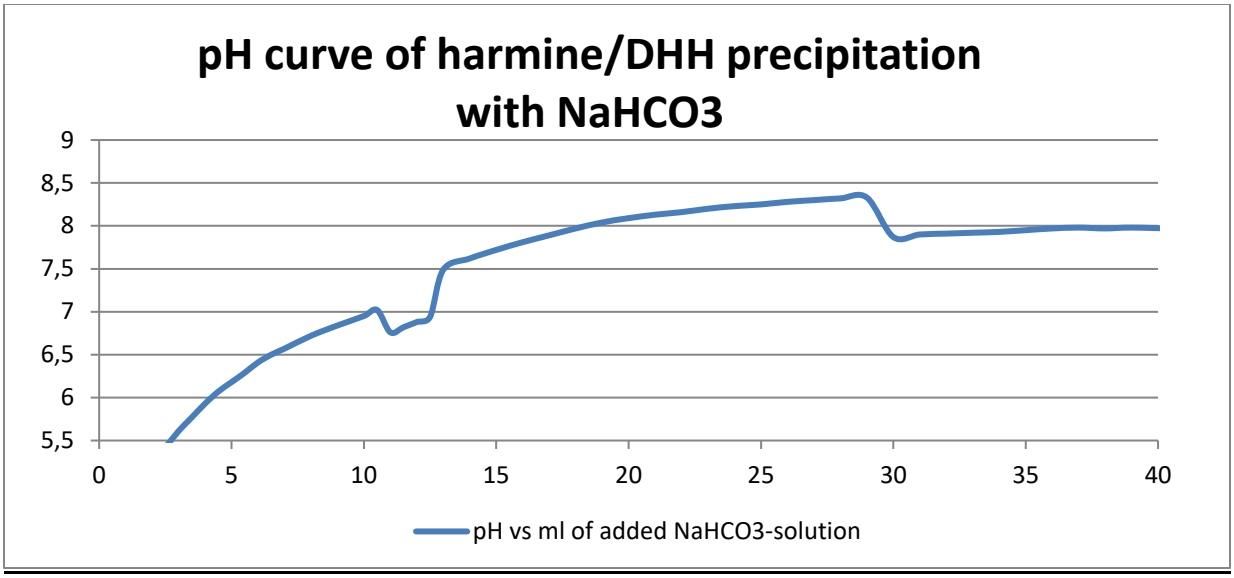


Image 11: Pure DHH prisms (RA5), sublimated from fraction RA4.

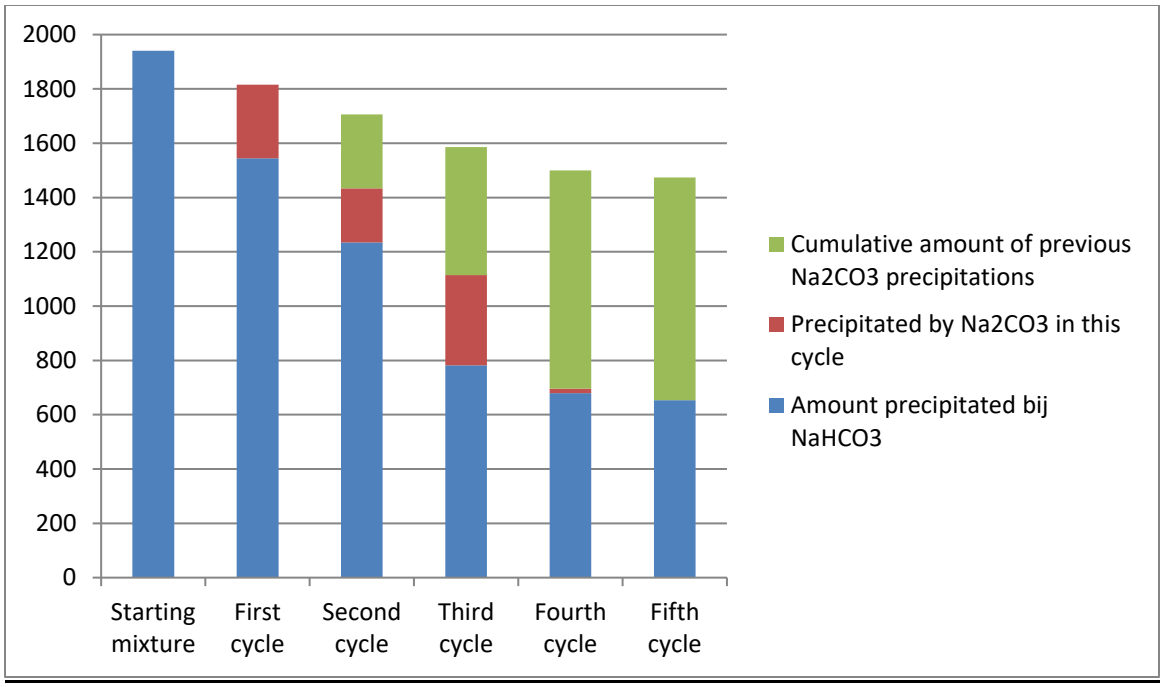
3.4 Using sodium bicarbonate as a base for precipitation

During the addition of $NaHCO_3$-solution, the pH of the solution progressively rose on standing, liberating CO2. This complicated exact pH measurement. The evolution of the solution's pH is drawn in **graph 3.4.1**.



Graph 3.4.1: The pH-variation during the addition of NaHCO3 is plotted. Between pH7-7,5 harmine precipitated, indicated by the transient pH-depression. Sodium bicarbonate was able to overcome the DHH-pH-peak around 8,4 which only led to partial precipitation of DHH for afterwards the bicarbonate could no more raise the pH above 8,0.

Because this first precipitation-cycle only led to partial separation of both alkaloids, 5 successive cycles were needed to bring about complete separation. **Graph 3.4.2** offers a visual representation of the complete process.



Graph 3.4.2 shows the evolution of residue masses in the course of 5 sodium bicarbonate/carbonate precipitation-cycles of a harmine-DHH mixture. Only after 5 cycles, no more DHH could be precipitated by Na_2CO_3.

Sample	RA1	RA2	RA3	RA4	RA5	RA6	RA7	RA8	RA9	RA10
Weight (mg)	1544	272	1234	200	782	332	680	16	654	0
Melting range °C (decomp.)	-	236-237,5	-	-	-	-	-	235-237,5	255,5-256,5	-

3.5 Conversion of DHH freebase to THH

Image 12 shows the change in the solution's color under UV-light when conversion of DHH to THH had taken place.

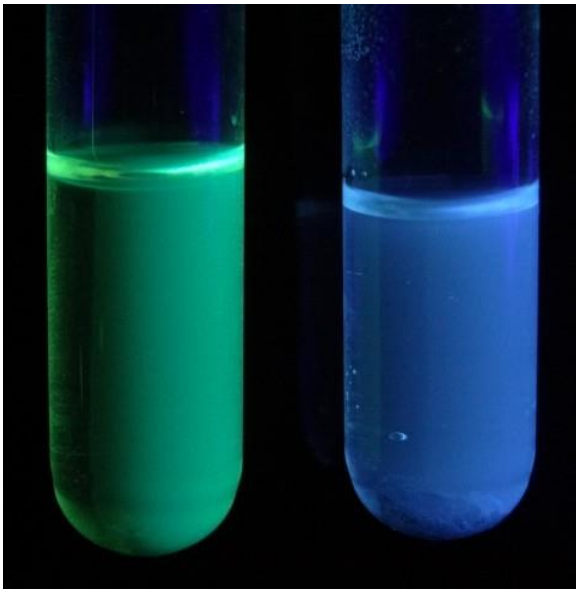


Image 12: UV-florescence of acidic DHH-acetate (left) and THH-acetate solutions (right). In both test-tubes, DHH freebase was dissolved in acetic acid 7%. To the test-tube on the right, zinc powder was added generating hydrogen gas (visible). Following the hydrogenation of DHH to THH, the solution's yellow-green color changed to blue.

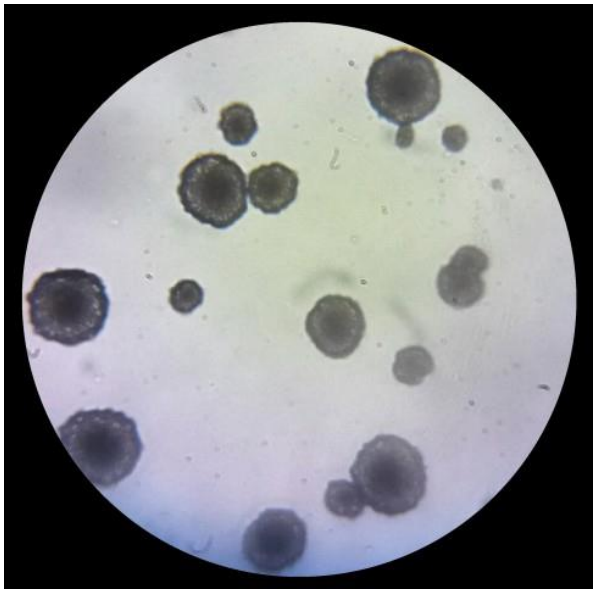


Image 13: THH precipitates out as spherical agglomerates of elongated crystals in residues RA3 and RA4.

Image 13 shows the microscopic appearance of precipitates RA3 and RA4. The THH crystallized in spherical clusters.

Image 14 shows the sublimated THH (RA5). It attaches as white/colorless needles to the sublimation flask. **Image 15** shows the same sample observed on a microscopic slide in dilute ammonia.

Image 14: Sublimated THH

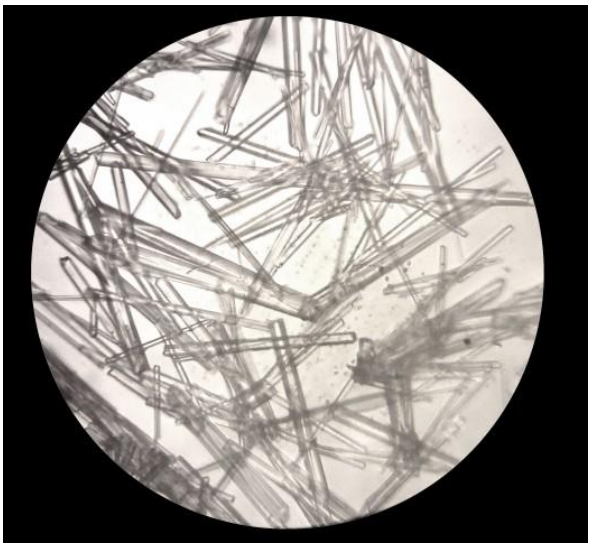
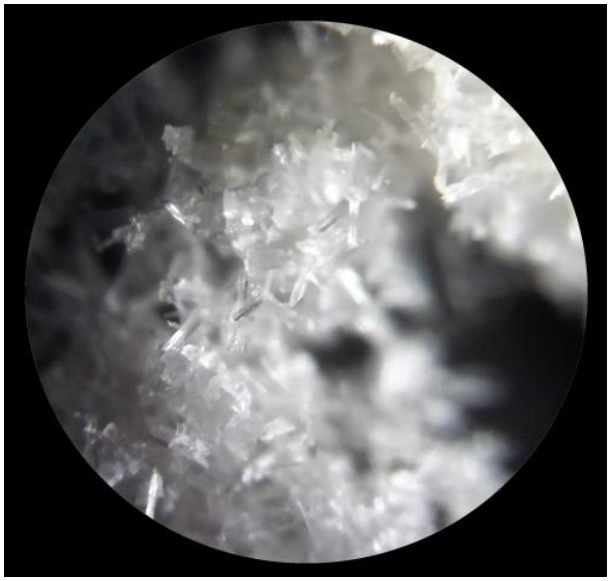


Image 15: Sublimated THH-crystals showing a uniform microscopic appearance as long 'sticks'

Image 16 and **image 17** show THH crystals that were formed by recrystallizing RA3 twice from ethanol 96% (RA6). When examined on a microscopic slide in dilute ammonia, their crystal shape is less uniform than that of sublimated THH. However, their narrow melting range confirms their comparable purity.



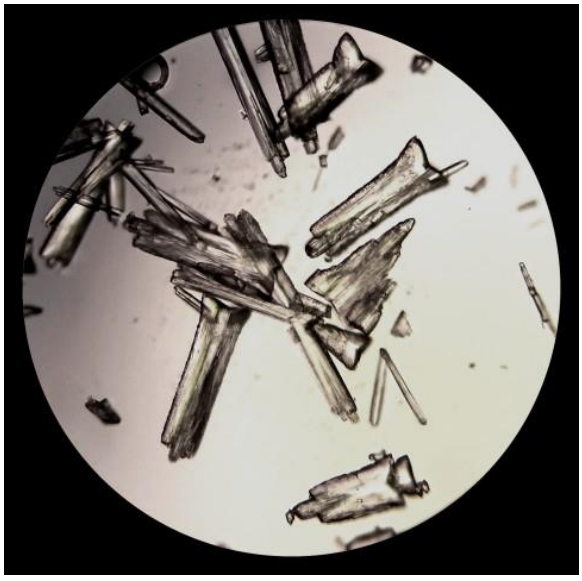


Image 16: THH recrystallized twice from ethanol (RA6)

Image 17: RA6: the crystal shape of recrystallized THH is more diverse than that of sublimated THH.

Sample	RA1	RA2	RA3	RA4	RA5	RA6
Weight mg	2060	70	1466	234	-	-
Melting range °C (decomp.)	-	-	190,5-192	191-192,5	193,5-194,5	193-194,5

3.6 Hydrogenation of harmine by zinc-acetic acid

91% of harmine was recovered in experiment 2.6.1 that served as a control for experiment 2.6.2 where 89% of harmine was recovered unreacted.

Microscopic examination of all reprecipitated residues showed an identical image of characteristic needle-shaped harmine crystals. Precipitates RA1 and RB3 showed the persistence of very small impurity-particles attached to the large harmine-needles. RB4 had very few of them. Impurities seem to serve as nucleation sites for the first crystals to form. Attached to these crystals, the impurities are filtered off with the first fraction(s), making the next fractions more pure. The color of RA1 and RB3 was off-white/brown while that of RA1 and RB3 was clear white. Also, the melting ranges of both confirmed the higher purity of RB4.

Sample	RA1	RB1	RB2	RB3	RB4
Weight mg	910	1236	0	778	112
Melting range °C (decomp.)	256-258	-	-	255,5-257	256,5-258

3.7 Zinc-acetic acid reduction of mixed harmine-DHH freebases and pH-specific separation with ammonia, $NaHCO_3$ and Na_2CO_3

Sample	R1A1	R2A1	R3A1	R1A2	R1B1	R1B2	R2B2	R2A2	R3A2
Weight mg	472	328	358	1974	398	404	-	402	412
Melting range °C (decomp.)	245-248	189-190,5	259-260	-	191-192,5	192-193,5	232-233,5	256-258	256-257

Residues R2A2 and R3A2 showed comparable melting ranges, indicating that precipitation from saturated NaCl-solution prior to freebasing has little added value.

Comparing the melting points of residues R1B1 and R2B2 to fractions RA5 and RA6 of experiment 2.5 indicates that the washed THH-precipitates already are quite pure.

4. DISCUSSION

The combined information of the above experiments can be applied in the development of low-tech protocols describing the isolation of harmine, DHH and THH starting from Peganum Harmala seed. These protocols are added as appendices.

Protocol 1 takes the seeds as starting material and finishes with a mixture of harmine and DHH freebases. For the initial extraction and selective separation of these alkaloids, Hasenfratz' method was followed [Hasenfratz, 1927]. It was confirmed that the acidic water extraction provided good yields: 7,6% total alkaloids after 4 extraction cycles. Clogging of filters was the main hindrance and could be overcome by giving the fine plant material enough time to settle down. Although not essential, a vacuum-filtration setup greatly reduced filtration times. When precipitating the hydrochloride salts, the decline in non-harmine/-DHH or -THH alkaloids that could be precipitated from the filtrate in each successive step, confirmed the selective separation of the β-carbolines from the other alkaloids. As was demonstrated, it is advisable to repeat this purification step 5 times to minimize the presence of other alkaloids that can, in the case of ingestion, be the cause of unwanted effects.

It was shown that sodium carbonate is strong enough to precipitate all three freebases. Considering its relative safety, this makes it the base of choice. However, the production of carbonic acid in its reactions gives rise to 2 disadvantages: excessive foaming in the initial extraction and the slow release of CO_2 that makes the pH difficult to measure in separation experiments. Therefore, ammonia was preferred in experiment 3.3 for exact measurement. Dilute ammonia is also preferred for washing the final products for it leaves no residue on evaporation. A sodium carbonate 0,5% solution can be used for washing as well without degrading the purity too much (cf. melting point of RA2, exp. 2.4).

While Hasenfratz then continued to use a microscopic method for the separation of harmine from DHH (protocol 2.2), this paper investigated a separation method based on pH-measurement. This revealed a most interesting characteristic of the alkaloids under study and led to the development of protocol 2.1. That DHH precipitates after harmine in the course of basification was already

known, but in this experiment the precise course of the pH-curve was drawn. It was seen that precipitation of the freebases leads to a concurrent depression of the mother liquor's pH. When one looks at graph 3.3.2 and 3.3.3, it becomes clear that between pH 6,6 and 7,5 (when precipitation of harmine is almost complete), the addition of base makes the pH rise quickly while the precipitation of harmine becomes negligible. Also, pH-depression is absent. This rapid pH-rise between pH 6,6-7,5 thus indicates the optimal moment for filtration of the harmine-fraction. The broad pH-interval makes it possible to use a basic pH-meter with a resolution of only 0,1pH. A second filtration was done right after the pH-peak at 7,7 had been overcome, giving a mixed harmine-DHH fraction. This fraction comprised only 2% of the total amount of precipitated base, making this method not only rapid and precise, but also very high yielding. On popular websites describing the separation of Syrian rue alkaloids, it is postulated that harmine can be separated from DHH on the basis of their different pKa, claiming that at a pH of 8,75, 92% of the harmine present and only 8% of DHH present will precipitate [Dmt-nexus 2016a, Erowid 2016a, Shroomery 2016, Mycotopia 2016]. The above experiment clearly contradicts this statement, showing that at pH 8,50 all of the DHH has already precipitated. This has important implications for harm-reduction, as DHH is a much stronger MAO-inhibitor than harmine [Herraiz et al., 2010].

Concerning the microscopic method of separation, **Images 1-5** show that the alkaloids under study can have different crystal shapes depending upon the pH, presence of contaminants and speed of recrystallization. Therefore, *in situ* reprecipitation of the freebase-fractions on the microscopic slide is advised to allow for a more standardized and precise identification of the alkaloids. This procedure was integrated in Protocol 2.2, thereby refining Hasenfratz' method.

The presence of THH could not be demonstrated in any of the extracts. Considering the results of experiment 2.5, one would expect THH to precipitate out after DHH. However, the melting range and microscopic appearance of the last fraction of experiment 2.3 (RA6) leave no doubt about it being DHH. This absence of THH could be explained by its concentration in the seeds being far lower than that of the other alkaloids [Herraiz et al., 2010] and/or by its selective removal by repeated conversion and basification in experiments 2.1 and 2.2.

Another claim that is made on dedicated websites is that sodium bicarbonate is unable to precipitate DHH, therefore making it possible to separate it from harmine in a one-step procedure [Dmt-nexus 2016a; Dmt-nexus 2016c]. This is contradicted by experiment 3.4. On graph 3.4.1 one sees that sodium bicarbonate is able to overcome the pH-peak preceding DHH precipitation. Afterwards however, it is not able to raise the pH again to such a level that all of the DHH precipitates. When successive sodium bicarbonate precipitation cycles are done, removing at every cycle a part of the DHH, it still takes five cycles to completely separate both alkaloids. Just as with the pH-metric method of separation, these results have important implications for harm-reduction. Because when only one separation cycle is done, a substantial amount of DHH is contained in the presumed harmine-fraction.
When the total yield of this method (protocol 2.3) is compared to the yield of the pH-metric method, it is clearly lower: 76% vs 91% recovery. The purity of the end-products, judged by their melting points, is comparable. It was also noted that this method gives a slightly different alkaloid recovery-ratio when compared to the pH-metric method: the relative recoveries for harmine/DHH are 49/51% (pH-metric) and 44/56% ($NaHCO_3$). Given its minimal requirements however, the yield of the bicarbonate method is acceptable.

Reduction of DHH to THH has been described by many authors [Erowid, 2016b]. In this current paper a method employing zinc and acetic acid is studied. Interestingly, this safe and simple reaction proceeded to give a high conversion and recovery yield (83%). Byproducts that precipitated could be easily removed by washing with dilute base. Considering its ease, yield and use of safe reagents, this method clearly deserves further investigation (protocol 3).

Next, it was investigated whether harmine could be reduced as well using this method. It was shown that even after long reaction times, 89% of the harmine could be recovered unreacted (versus 91% in the control experiment). Moreover, no precipitation at higher pH's was observed, confirming the absence both of DHH and THH. This observation is interesting because it puts to question the hypothesis of Callaway that THH might be formed from harmine throughout the

preparation process of ayahuasca [Callaway, 2005]. Only the conversion of DHH to THH seems plausible in the light of the present results.

Building upon the results of both conversion experiments, a low-tech approach to obtaining harmine and THH from a mixed extract containing harmine and DHH was applied in experiment 2.7 (protocol 4). The results confirm the possibility of converting the end-product of experiment 2.2 (protocol 1) without need of any equipment into separate harmine and THH with good yields (both around 80%). In contrast to the separation of harmine and DHH, sodium bicarbonate can now be applied to separate harmine and THH in a single step, for no THH could be precipitated by it. If protocols 1 and 4 are successively applied to Peganum Harmala seeds, one is able to obtain pure harmine and THH by only using a filtration setup, vinegar, salt, sodium bicarbonate and zinc-powder. Interestingly, the ratio of alkaloids obtained by this method is comparable to the one found in ayahuasca brews, being about equally high in harmine and THH while being low in DHH [Callaway, 2005].

Notwithstanding the simplicity of all these methods, the purity of the alkaloids was confirmed in three ways. First, the crystalline residues were microscopically observed to contain no amorphous material nor differently shaped crystals. Secondly, the measured melting ranges of the end-products were quite narrow, even considering the use of a Thiele-tube and the decomposition that all three alkaloids go through near their melting range. Thirdly, the melting ranges of the washed and/or recrystallized samples were compared to those of the respective sublimated alkaloid. They were shown to be near-identical and to correspond to values found in literature: harmine 257-258°C (Telezhenetskaya, 1977); dihydroharmine 236-238°C (Ghosal S, Mazumber UK, Bhattahcharaya SK, 1972); tetrahydroharmine 187-190°C (Shulgin, Shulgin 1997), 190-195°C (Bernauer, 1964) ; tetrahydroharmine hydrochloride 232-234°C (Shulgin S, Shulgin A, 1997).

5. CONCLUSION

This study provides safe and effective protocols for the extraction, separation, conversion and purification of harmine, DHH and THH from Peganum Harmala seed using minimal technical equipment and accessible reagents. This would make them applicable in traditional and other low-tech contexts, providing more specific pharmacological action and harm-reduction to users.

A synthesis route for THH by hydrogenation of DHH using zinc-acetic acid was found to be effective with 83% yield. Also, a rapid, precise and high-yielding method for bulk separation of harmine and DHH using basic pH-metry was developed.

6. ACKNOWLEDGEMENTS

The author wants to thank anonymous reasearchers at www.dmt-nexus.me for pointing out that sodium carbonate can not be used as a base in the synthesis and isolation of THH due to insoluble zinc-salts precipitating out. Therefore the protocols in this preprint-article were updated.

7. ABOUT THE AUTHOR

Frederick Van Der Sypt, M.D. is currently working as an addiction specialist in the residential treatment centers for substance abuse of ‘De Sleutel’. He is a lecturer at the Expertise-center for Alcohol and Drug-related disorders (VAD, Brussels), teaching diverse topics on substance use disorders and psychofarmacology. His fields of interest are harm reduction, new psychoactive substances and the therapeutic potential of ethnopsychopharmacology.

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9. APPENDICES

Protocols

PROTOCOL 1

From Peganum Harmala seed to a mixture of harmine and DHH freebase

Boil seeds in acidified water for 1h
|
filter and collect filtrate (repeat 4-5 times)
|
discard seed mash
|
allow 12-24h for sedimentation of the combined filtrates and run supernatant through fine filterpaper, discard residue
|
add excess base (Na_2CO_3 or other) to the filtrates, filter and discard filtrate
|
dissolve residue in hot dilute acetic acid and add an equal volume of hot saturated NaCl-solution
|
let cool slowly for 12h and filter
|
dissolve residue in hot water and add an equal volume of hot saturated NaCl-solution
|
let cool slowly for 12h and filter (repeat last 2 steps 4 times)
|
dissolve residue in hot water, add excess base (Na_2CO_3 or other) and filter
|
wash residue with ammonia 3% or Na_2CO_3 solution 0,5%

PROTOCOL 2

SEPARATING A MIXTURE OF HARMINE AND DHH

2.1 When a pH-meter with accuracy of 0,1pH is available	**2.2** When a microscope is available	**2.3** When neither a pH-meter nor a microscope is available
Dissolve end-product of Protocol 1 in dilute acetic acid	Dissolve end-product of Protocol 1 in dilute acetic acid	Dissolve end-product of Protocol 1 in dilute acetic acid
Slowly add base (Na_2CO_3 or other), precipitate will form and the pH will start lowering again when it has reached pH 7,2	Slowly add base until precipitation starts, filter and examine crystals under microscope	Add excess of concentrated $NaHCO_3$ solution, filter (residue=**mixed fraction**)
Keep adding base until the pH rises again to pH7,5 without lowering, filter (**Harmine-fraction**)	Repeat previous step until plates (or, in the case of reprecipitation *in situ* on the microscopic slide, leaf-like patterns) are seen together with the harmine crystals that precipitate first	Add excess Na_2CO_3 to filtrate, filter and keep apart (**DHH-fraction**)
Again add base to mother liquor in small increments until precipitation occurs (+/-pH 7,7), filter and discard residue (**mixed fraction**)	discard last fraction (**mixed-fraction**). All previous precipitates form the **harmine-fraction**	Redissolve mixed fraction in dilute acetic acid and repeat steps 2-4 until the addition of Na_2CO_3 causes no more precipitation
Add excess base to raise the pH of the mother liquor to minimum 8,5 and filter off precipitate (**DHH-fraction**)	Add excess base to precipitate **DHH-fraction** and filter	Last NaHCO3-precipitated residue= **harmine fraction**; Combine DHH-fractions
Purify the residues by redissolving them separately in acetic acid and freebasing them again with strong ammonia or Na_2CO_3 solution	Purify the residues by redissolving them separately in acetic acid and freebasing them again with strong ammonia or Na_2CO_3 solution	Purify the residues by redissolving them separately in acetic acid and freebasing them again with strong ammonia or Na_2CO_3 solution
wash residues with ammonia 3% or dilute Na_2CO_3 solution 0,5%	wash residues with ammonia 3% or dilute Na_2CO_3 solution 0,5%	Wash residues with ammonia 3% or dilute Na_2CO_3 solution 0,5%

PROTOCOL 3

Converting DHH into THH using zinc-acetic acid

Dissolve DHH in acetic acid 7%, add zinc powder and let react for 12h
(optional: verify with UV-light that conversion has taken place)
|
filter off unreacted zinc and add ammonia until no more precipitation is observed
|
Filter and wash residue with ammonia 3%

PROTOCOL 4

Converting a mixture of harmine and DHH in separate harmine and THH

No pH-meter or microscope needed

Dissolve end product of protocol 1 in vinegar 7%
|
Add zinc powder and let react for 12h at room temperature
(optional: verify with UV-light that conversion has taken place)
|
Filter, discard unreacted zinc and add an excess of $NaHCO_3$ to the mother liquor, thereby precipitating the **harmine-fraction**, filter
|
add excess ammonia to the filtrate to precipitate the **THH-fraction**, filter and discard filtrate
|
clean up **harmine- and THH-fraction** separately by redissolving them in very dilute vinegar and reprecipitating them with a minimum amount of ammonia
|
Filter and wash both residues with ammonia 3%

A harm-reduction approach to the isolation of harmine and its hydrogenated derivatives from Peganum Harmala seed in low-tech settings

Frederick A.P. Van Der Sypt, M.D.

Corresponding Author:

Frederick Van Der Sypt

Geitstraat 83, Gent, 9000-Belgium

Email address: frederick.vandersypt@fracarita.org

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This article currently has the form of a preprint, waiting to be peer-reviewed. Interested journals are invited to contact the author at the aforementioned email address.